SUPPORTING INFORMATION

A Complementary Palette of NanoCluster Beacons

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I. NanoCluster Beacons in C-, P-, and S-series

Table S1. Oligonucleotide sequences of NCBs in C-, S-, and P-series. In the NC probes, the polycytosine heads are shown in blue, the linker in purple, the substituted cytosine in green, and the hybridization sequence in black. The G-rich enhancer sequence in the common enhancer probe is shown in red.

Name	DNA Sequence (5'→3')
	C-Series NC Probe
C_{2-2}	**CC** TTAAT **CC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{3-3}	**CCC** TTAAT **CCC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{3-4}	**CCC** TTAAT **CCCC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{4-3}	**CCCC** TTAAT **CCC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{4-4}	**CCCC** TTAAT **CCCC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{5-5}	**CCCCC**TTAAT **CCCCC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{6-6}	**CCCCCC**TTAAT **CCCCCC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{7-7}	**CCCCCCC** TTAAT **CCCCCCC**TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
C_{8-8}	**CCCCCCCC** TTAAT **CCCCCCCC** TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
	P-Series NC Probe
P1	CCC **C**TAAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
P2	CCC T**C**AAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
P3	CCC TT**C**AT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
P4	CCC TTA**C**T CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
P5	CCC TTAA**C** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
	S-Series NC Probe
S10	CCC **TTAATTAATT** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S9	CCC **TTAATTAAT** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S8	CCC **TTAATTAA** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S7	CCC **TTAATTA** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S6	CCC **TTAATT** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S5	CCC **TTAAT** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S4	CCC **TTAA** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S3	CCC **TTA** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S2	CCC **TT** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S1	CCC **T** CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
S0	CCC CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
	Common Enhancer Probe
	ATT AAT AAA TAA TAT TTA AAA TTT ATT ATA GGGTGGGGTGGGGTGGGG

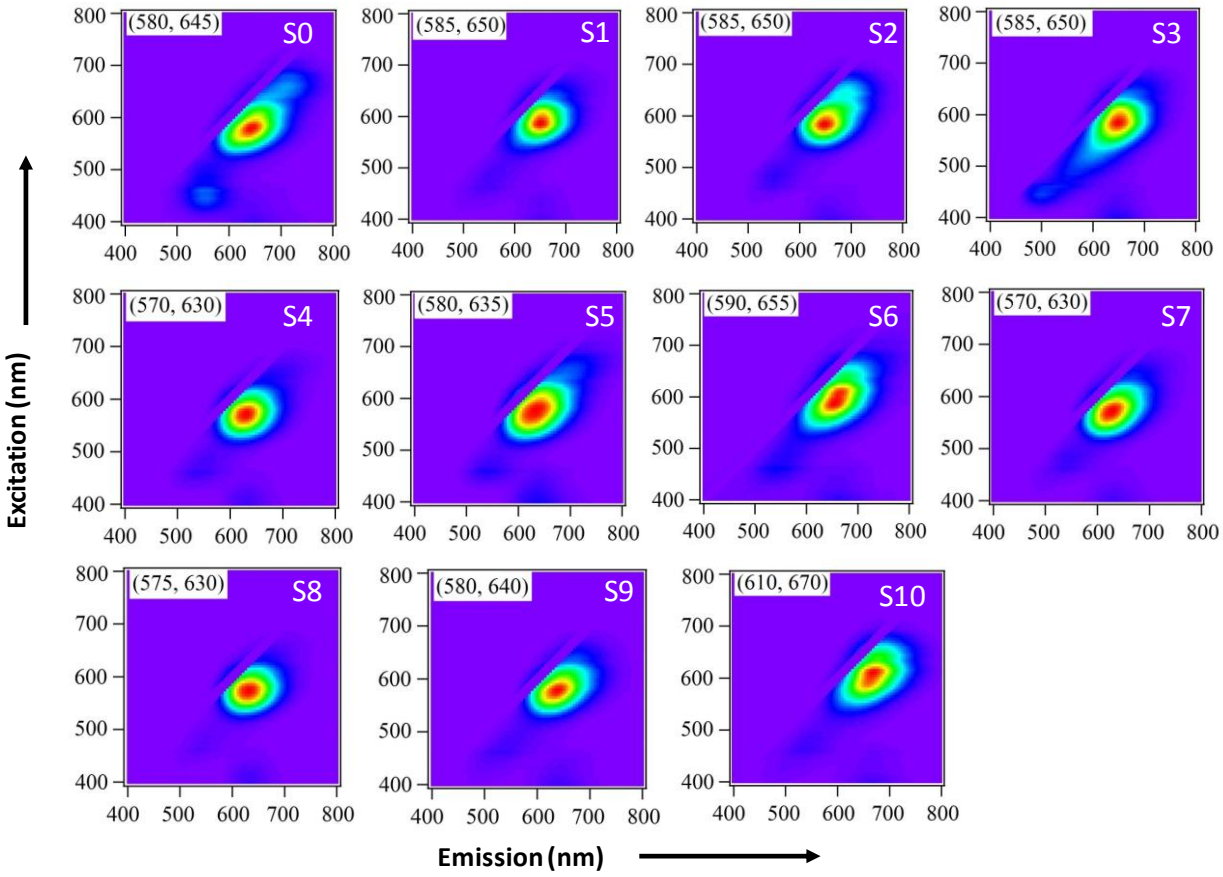
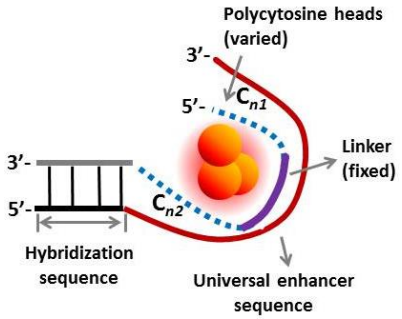


Figure S1. Normalized 2D fluorescence contour plots of NCBs in S-series. The peaks of these 2D spectra are nearly identical and the emission patterns are relatively pure (as compared to C- and P-series). However, these spectra differ in the symmetry of their spectral profiles. S1-4 & S8 have symmetric profiles (eccentricity < 0.66) while S0, S5-7 & S9-10 have asymmetric profiles (eccentricity ≥ 0.66).

C-series Design

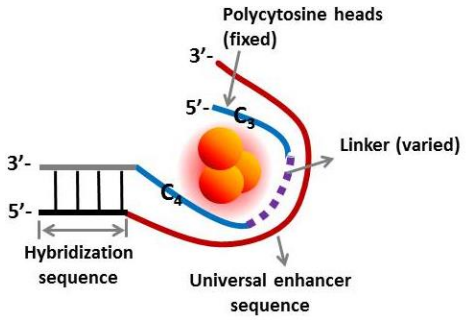


Name	Sequence	No. of polycytosines	Eccentricity of major spectral peaks	Major emission peak (nm)	FI_{max} of major peak (a. u.)	FI_{max} of 2º peak (a. u.)	Spectral purity
$C_{2\text{-}2}$	ATATCCTAATTCC-5′ TATAGGGTGGGGTGGGGTGGGG-3′	4	0.53	650	0.93	0.06 (6%)	relatively pure
$C_{3\text{-}3}$	ATATCCCTAATTCCC TATAGGGTGGGGTGGGGTGGGG	6	0.78	695	2.3	2.0 (87%)	not pure
$C_{3\text{-}4}$	ATATCCCCTAATTCCC TATAGGGTGGGGTGGGGTGGGG	7	0.76	635	8.0	1.2 (15%)	relatively pure
$C_{4\text{-}4}$	ATATCCCCTAATTCCCC TATAGGGTGGGGTGGGGTGGGG	8	0.79	635	1.3	0.47 (36%)	not pure
$C_{5\text{-}5}$	ATATCCCCCTAATTCCCCC TATAGGGTGGGGTGGGGTGGGG	10	0.57	585	10	1.7 (17 %)	relatively pure
$C_{6\text{-}6}$	ATATCCCCCCTAATTCCCCCC TATAGGGTGGGGTGGGGTGGGG	12	0.64	590	12	5.6 (47%)	not pure
$C_{7\text{-}7}$	ATATCCCCCCCTAATTCCCCCCC TATAGGGTGGGGTGGGGTGGGG	14	0.74	590	3.6	3.2 (89%)	not pure
$C_{8\text{-}8}$	ATATCCCCCCCCTAATTCCCCCCCC TATAGGGTGGGGTGGGGTGGGG	16	0.81	555	7.4	0.45 (6%)	relatively pure

Hybridization sequence Polycytosine heads under investigation

Figure S2. Schematic representation of NCBs in C-series. The C-series NCBs consist of a set of NC probes differing in the size of polycytosine heads but having an identical linker sequence between the heads. **Upper:** Schematic diagram showing the conceptual arrangement of the polycytosine heads, linker, enhancer and hybridization sequences in the C-series (not drawn to scale). **Lower:** Alignment of the cluster-nucleation sequence with respect to the G-rich enhancer sequence, number of cytosines in the cluster-nucleation sequence, the eccentricity of major spectral peaks, the major emission peaks and the spectral purity of the C-series NCBs. A 2D fluorescence contour plot is defined as "relatively spectrally pure" if the fluorescence intensity of its secondary (2º) peak is less than 25% of its major peak intensity. $C_{3\text{-}4}$ represents a design with a C_3 and a C_4 polycytosine heads. $C_{3\text{-}4}$ is the first NCB reported,[1] which serves as the gold standard in this report.

S-series Design

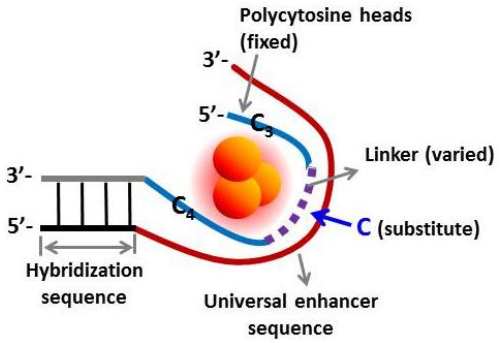


Name	Sequence	Eccentricity of major spectral peaks	Major emission peak (nm)	FI_{max} of major peak (a. u.)	FI_{max} of 2° peak (a. u.)	Spectral purity
S0	ATAT CCCCCCC-5′ TATA GGGTGGGGTGGGGTGGGG-3′	0.80	645	3.7	0.89 (24%)	relatively pure
S1	ATAT CCCCTCCC TATA GGGTGGGGTGGGGTGGGG	0.52	650	4.1	0.52 (13%)	relatively pure
S2	ATAT CCCCTTCCC TATA GGGTGGGGTGGGGTGGGG	0.59	650	6.9	2.3 (33%)	not pure
S3	ATAT CCCCATTCCC TATA GGGTGGGGTGGGGTGGGG	0.59	650	9.1	2.2 (24%)	relatively pure
S4	ATAT CCCCAATTCCC TATA GGGTGGGGTGGGGTGGGG	0.61	630	8.1	0.80 (10%)	relatively pure
S5	ATAT CCCCTAATTCCC TATA GGGTGGGGTGGGGTGGGG	0.76	640	8.0	1.2 (15%)	relatively pure
S6	ATAT CCCCTTAATTCCC TATA GGGTGGGGTGGGGTGGGG	0.79	655	2.5	0.42 (17%)	relatively pure
S7	ATAT CCCCATTAATTCCC TATA GGGTGGGGTGGGGTGGGG	0.74	630	7.1	0.91 (13%)	relatively pure
S8	ATAT CCCCAATTAATTCCC TATA GGGTGGGGTGGGGTGGGG	0.59	630	11	1.3 (12%)	relatively pure
S9	ATAT CCCCTAATTAATTCCC TATA GGGTGGGGTGGGGTGGGG	0.78	640	5.3	0.73 (14%)	relatively pure
S10	ATAT CCCCTTAATTAATTCCC TATA GGGTGGGGTGGGGTGGGG	0.73	670	3.8	0.42 (11%)	relatively pure

Hybridization sequence Linker(s) under investigation

Figure S3. Schematic representation of NCBs in S-series. The S-series NCBs consist of a set of NC probes differing in the linker length but having identical polycytosine heads. **Upper:** Schematic diagram showing the conceptual arrangement of the polycytosine heads, linker, enhancer and hybridization sequences in the S-series (not drawn to scale). **Lower:** Alignment of the cluster-nucleation sequence with respect to the G-rich enhancer sequence, the eccentricity of major spectral peaks, the major emission peaks and the spectral purity of the S-series NCBs. S3 denotes design with a 3-nt long linker. Here S5 is identical to the gold standard $C_{3\text{-}4}$.

P-series Design



Name	Sequence	Eccentricity of major spectral peaks	Major emission peak (nm)	FI_{max} of major peak (a. u.)	FI_{max} of 2° peak (a. u.)	Spectral purity
P1	ATAT CCCCTAATCCCC-5′ TAT AGGGTGGGGTGGGGTGGGG-3′	0.86	640	2.5	0.89 (36%)	not pure
P2	ATAT CCCCTAACTCCC TAT AGGGTGGGGTGGGGTGGGG	0.87	630	2.3	0.64 (28%)	not pure
P3	ATAT CCCCTACTTCCC TAT AGGGTGGGGTGGGGTGGGG	0.58	640	9.1	1.2 (13%)	relatively pure
P4	ATAT CCCCTCATTCCC TAT AGGGTGGGGTGGGGTGGGG	0.82	620	1.9	0.85 (45%)	not pure
P5	ATAT CCCCCAATTCCC TAT AGGGTGGGGTGGGGTGGGG	0.66	635	4.5	4.2 (93%)	not pure

Hybridization sequence Cytosine substitution under investigation

Figure S4. Schematic representation of NCBs in P-series. The P-series NCBs consist of a set of NC probes differing in the cytosine substitution location in the linker. **Upper:** Schematic diagram showing the conceptual arrangement of the polycytosine heads, linker, enhancer and hybridization sequences, and the location of the substituted cytosine in the linker (not drawn to scale). **Lower:** Alignment of the cluster-nucleation sequence with respect to the G-rich enhancer sequence, the eccentricity of major spectral peaks, the major emission peaks and the spectral purity of the P-series NCBs. P3 denotes a design with the 3rd linker nucleotide being substituted with a cytosine.

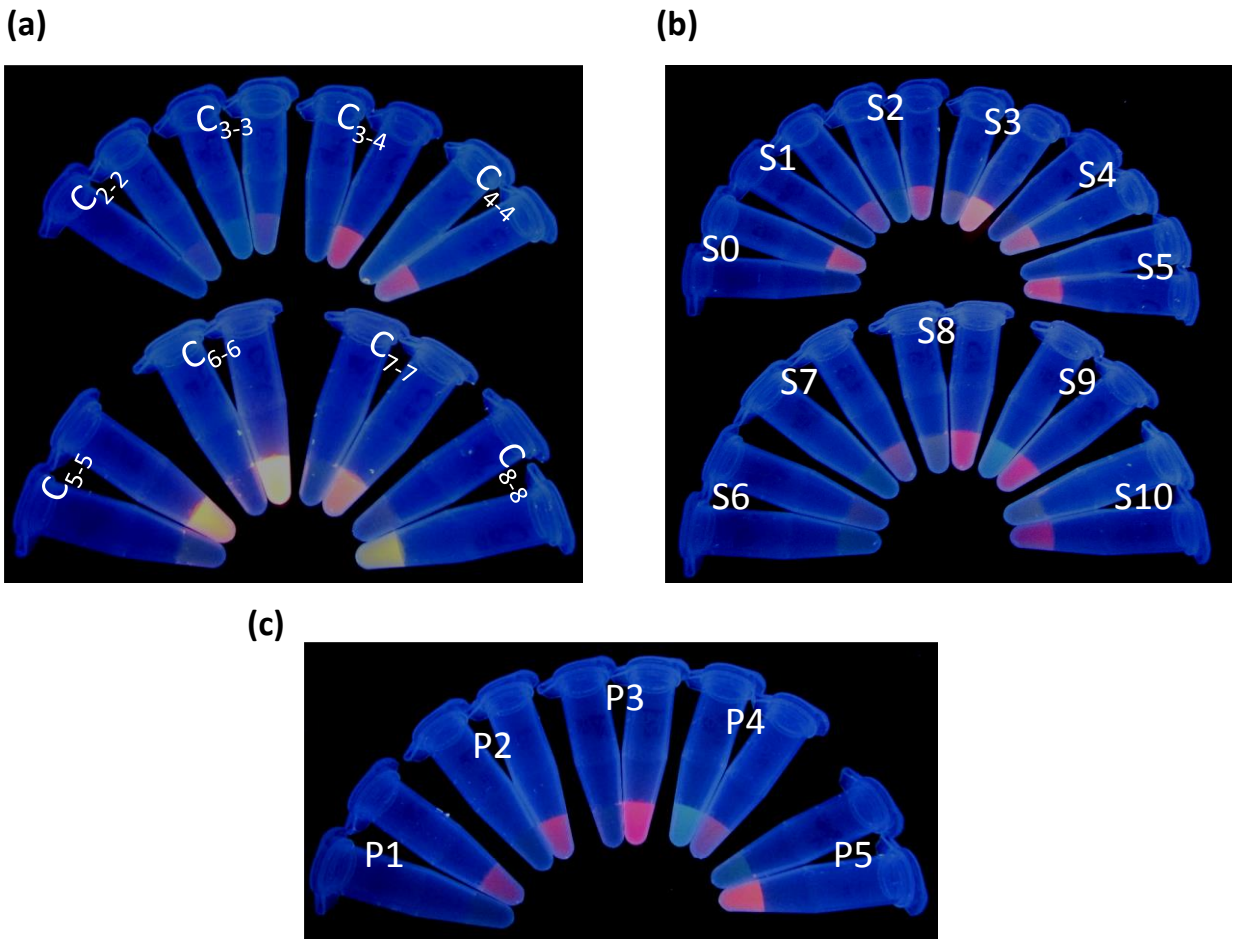


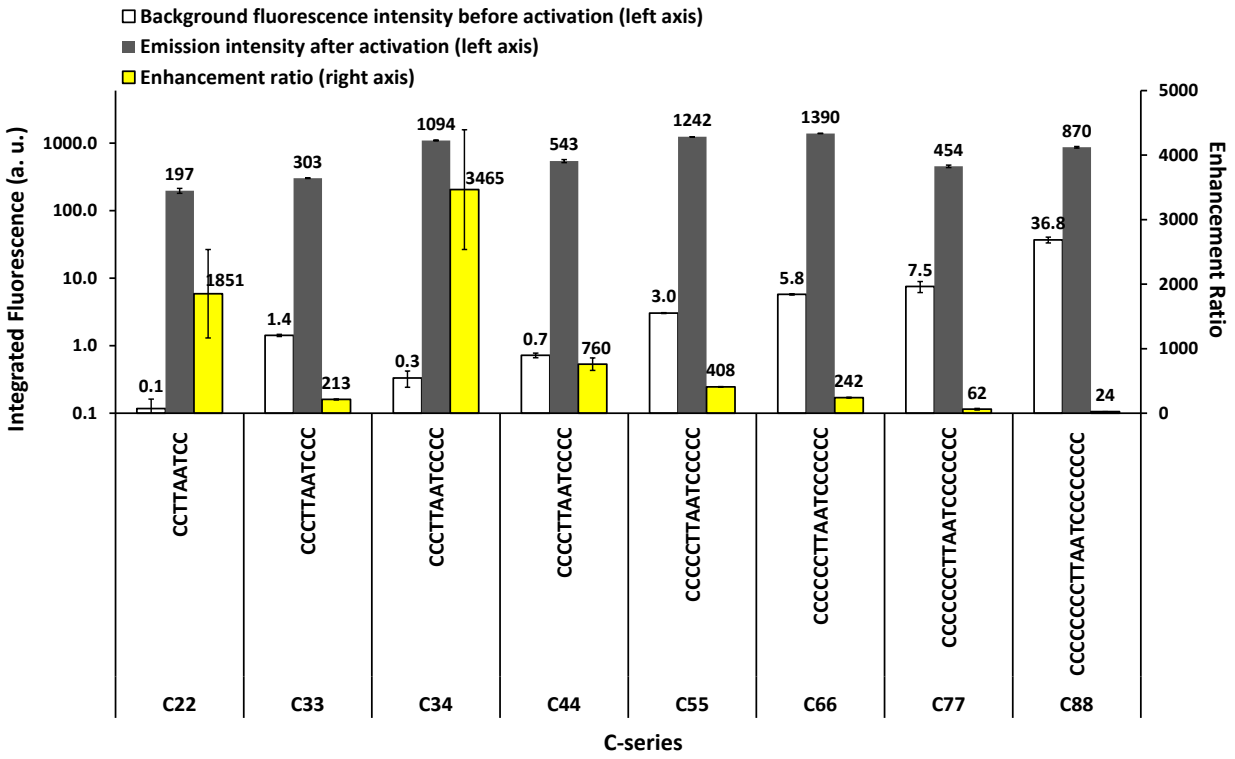
Figure S5. Color photos of NCBs in (a) C-series, (b) S-series and (c) P-series. For each sample subset, the left vial contained the inactivated NCB (*i.e.* NC probe only) while the right vial contained the activated NCB (*i.e.* the duplex). Photos were taken 24 hrs (samples stored at -20 °C) after the fluorescence measurements were completed. λ_{ex} = 365 nm. NCBs were prepared at 15 μM concentration.

Table S2. Enhancement ratios of S-series NCBs. The enhancement ratios were estimated based on the 1D scans using a fixed excitation wavelength of 580 nm and calculated as $(I_{activated}-I_{buffer})/(I_{inactivated}-I_{buffer})$. Fluorescence intensities before and after activation were integrated from 595 nm to 740 nm. All emission intensities after activation were compared to the gold standard, S5 (also $C_{3\text{-}4}$), whose relative intensity is set to 100%.

S-series	Sequence variation (5' --> 3')	Background fluorescence intensity before activation (a. u.)	Emission intensity after activation (a. u.)	Enhancement ratio
S0	CCCCCCC	8.7 ± 0.04	498 ± 7 (46 %)	57 ± 1
S1	CCCTCCCC	2.2 ± 0.02	778 ± 39 (71 %)	356 ± 15
S2	CCCTTCCCC	1.5 ± 0.04	709 ± 22 (65 %)	479 ± 28
S3	CCCTTACCCC	3.0 ± 0.08	984 ± 21 (90 %)	324 ± 7
S4	CCCTTAACCCC	1.2 ± 0.11	992 ± 34 (91 %)	824 ± 105
S5	CCCTTAATCCCC	0.3 ± 0.09	1094 ± 18 (100 %)	3465 ± 928
S6	CCCTTAATTCCCC	1.1 ± 0.07	266 ± 19 (24 %)	238 ± 17
S7	CCCTTAATTACCCC	1.3 ± 0.20	874 ± 87 (80 %)	659 ± 87
S8	CCCTTAATTAACCCC	1.9 ± 0.23	966 ± 5 (88 %)	527 ± 73
S9	CCCTTAATTAATCCCC	1.8 ± 0.05	724 ± 11 (66 %)	398 ± 17
S10	CCCTTAATTAATTCCCC	1.5 ± 0.07	339 ± 3 (31 %)	219 ± 11

Table S3. Enhancement ratios of P-series NCBs. The enhancement ratios were estimated based on the 1D scans using a fixed excitation wavelength of 580 nm and calculated as $(I_{activated}-I_{buffer})/(I_{inactivated}-I_{buffer})$. Fluorescence intensities before and after activation were integrated from 595 nm to 740 nm. All emission intensities after activation were compared to the gold standard, S5 (also $C_{3\text{-}4}$), whose relative intensity is set to 100%.

P-series	Sequence variation (5' --> 3')	Background fluorescence intensity before activation (a. u.)	Emission intensity after activation (a. u.)	Enhancement ratio
P1	CCC CTAAT CCCC	1.8 ± 0.02	297 ± 14 (27 %)	161 ± 9
P2	CCC TCAAT CCCC	0.8 ± 0.09	337 ± 25 (31 %)	413 ± 79
P3	CCC TTCAT CCCC	1.5 ± 0.09	851 ± 14 (78 %)	579 ± 27
P4	CCC TTACT CCCC	2.1 ± 0.02	275 ± 7 (25 %)	132 ± 5
P5	CCC TTAAC CCCC	2.4 ± 0.04	662 ± 33 (61 %)	277 ± 18



C-series	λ_{ex} (nm)	λ_{em} (nm)	Integration range (nm)
$C_{2\text{-}2}$	580	650	595-740
$C_{3\text{-}3}$	645	695	655-800
$C_{3\text{-}4}$	580	640	595-740
$C_{4\text{-}4}$	580	635	595-740
$C_{5\text{-}5}$	525	585	535-680
$C_{6\text{-}6}$	525	590	535-680
$C_{7\text{-}7}$	525	590	535-680
$C_{8\text{-}8}$	460	555	470-615

Figure S6. Enhancement ratios of C-series NCBs. Because the emission peak varies greatly in C-series experiment, the brightness and enhancement ratios of the NCB were compared using the maximum excitation depicted on each design (see Table right below Figure S6). The fluorescence intensities before and after activation were integrated at different spectral bands but with a fixed bandwidth of 145 nm.

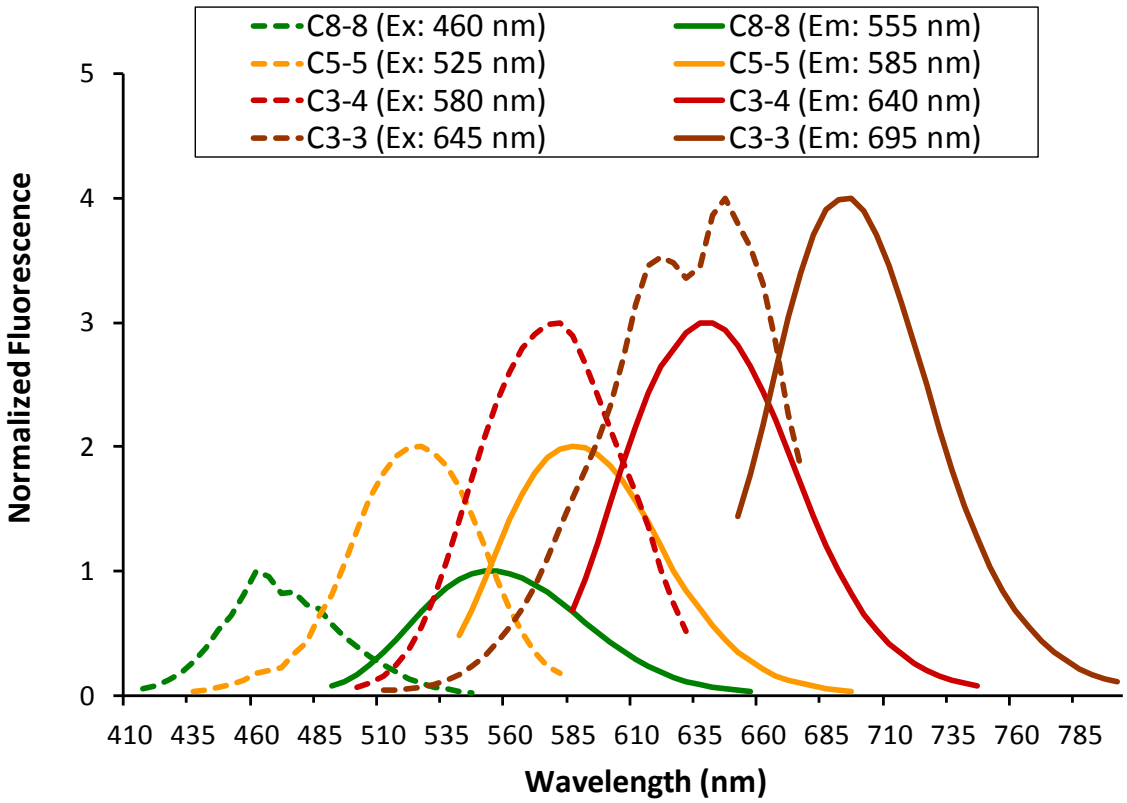


Figure S7. Normalized excitation/emission spectra of $C_{8\text{-}8}$, $C_{5\text{-}5}$, $C_{3\text{-}4}$ and $C_{3\text{-}3}$. Dashed lines represent the excitation spectra while solid lines represent the emission spectra. Normalization scale is set differently to ease visualization.

II. NanoCluster Beacons in the 32 Linker Variation Experiments

In this separate set of experiments, we varied the composition of the linker (5 nt long and made of only T or A) and generated 32 (a permutation of 2^5) distinct cluster-nucleation sequences for investigation (Tables S4 and S5). We found that the linker composition could have significant influence on the enhancement ratio (Figure S8), but not much effect on the activation color (Figure S9).

Table S4. DNA sequences of 32 NC probes. Here the polycytosine heads are shown in blue, the linker in purple, and the hybridization sequence in black. No. 9 NCB (highlighted in yellow) here is the gold standard.

Number	DNA Sequence (5'→3')
	NC Probe
1	CCC TTTTT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
2	CCC ATTTT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
3	CCC TATTT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
4	CCC TTATT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
5	CCC TTTAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
6	CCC TTTTA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
7	CCC AATTT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
8	CCC TAATT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
9	CCC TTAAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
10	CCC TTTAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
11	CCC ATATT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
12	CCC TATAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
13	CCC TTATA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
14	CCC ATTAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
15	CCC TATTA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
16	CCC ATTTA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
17	CCC TTAAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
18	CCC ATTAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
19	CCC AATTA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
20	CCC AAATT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
21	CCC TATAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
22	CCC ATATA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
23	CCC AATAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
24	CCC TAATA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
25	CCC ATAAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
26	CCC TAAAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
27	CCC AAAAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
28	CCC TAAAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
29	CCC ATAAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
30	CCC AATAA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
31	CCC AAATA CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
32	CCC AAAAT CCCC TAT AAT AAA TTT TAA ATA TTA TTT ATT AAT
	Common Enhancer Probe
	ATT AAT AAA TAA TAT TTA AAA TTT ATT ATA GGGTGGGGTGGGGTGGGG

Table S5. Enhancement ratios of 32 NCBs. Fluorescence was measured using a Varian Cary Eclipse Fluorescence Spectrophotometer. Emission intensities were integrated from 595 nm to 800 nm, under 580 nm excitation. The NC probe:Ag^{+}:$NaBH_4$ molar ratio used here is 1:12:12.

Number	Linker composition	Background Fluorescence Before Activation (a.u)	Emission Intensity After Activation (a.u)	Enhancement Ratio
1	**TTTTT**	16.4	10207.7	623
2	**ATTTT**	52.1	18483.2	355
3	**TATTT**	19.2	9730.4	507
4	**TTATT**	11.4	7528.8	663
5	**TTTAT**	12.0	8462.3	706
6	**TTTTA**	28.6	17908.6	627
7	**AATTT**	116.1	18034.4	155
8	**TAATT**	17.3	11334.9	655
9	**TTAAT**	10.1	15225.0	1511
10	**TTTAA**	23.2	20164.1	871
11	**ATATT**	101.9	8808.9	86
12	**TATAT**	43.8	9793.1	224
13	**TTATA**	58.8	11185.8	190
14	**ATTAT**	74.9	14879.0	199
15	**TATTA**	99.9	11048.8	111
16	**ATTTA**	176.1	21050.0	120
17	**TTAAA**	35.7	15886.8	445
18	**ATTAA**	165.6	25700.3	155
19	**AATTA**	178.1	10090.1	57
20	**AAATT**	204.8	9416.7	46
21	**TATAA**	101.2	15847.3	157
22	**ATATA**	389.3	9691.6	25
23	**AATAT**	153.6	16500.0	107
24	**TAATA**	399.9	9305.2	23
25	**ATAAT**	140.3	11741.2	84
26	**TAAAT**	50.0	6266.1	125
27	**AAAAA**	632.5	13386.0	21
28	**TAAAA**	254.6	14621.4	57
29	**ATAAA**	421.9	19591.5	46
30	**AATAA**	472.2	11949.5	25
31	**AAATA**	1340.1	16284.0	12
32	**AAAAT**	275.2	10744.0	39

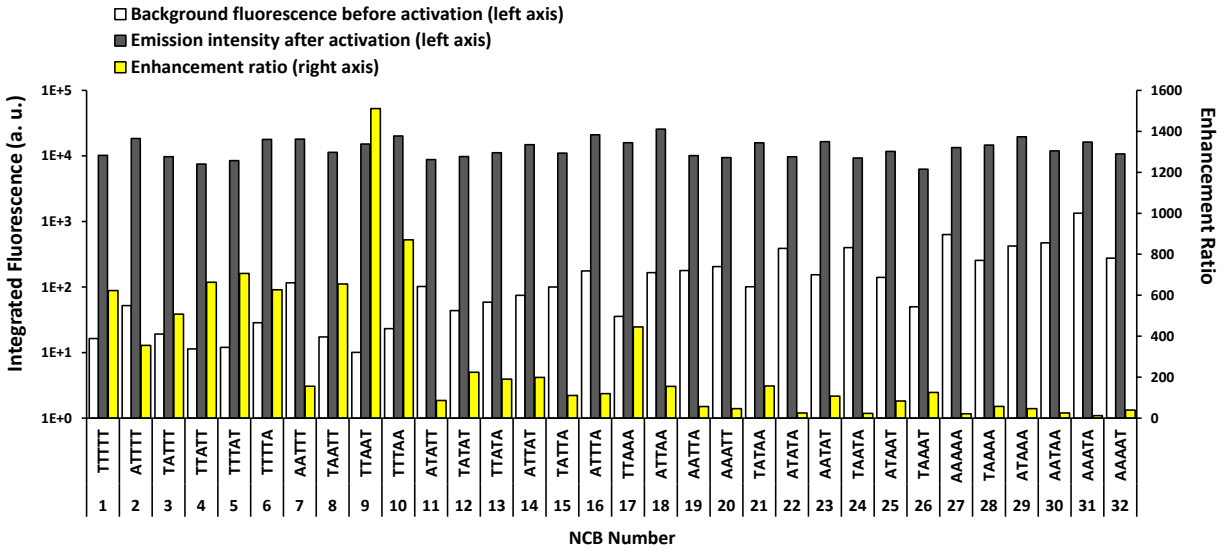


Figure S8. Enhancement ratios of 32 NCBs. Fluorescence was measured using a Varian Cary Eclipse Fluorescence Spectrophotometer. Emission intensities were integrated from 595 nm to 800 nm, under 580 nm excitation. No. 9 NCB is the gold standard (also $C_{3\text{-}4}$ and S5).

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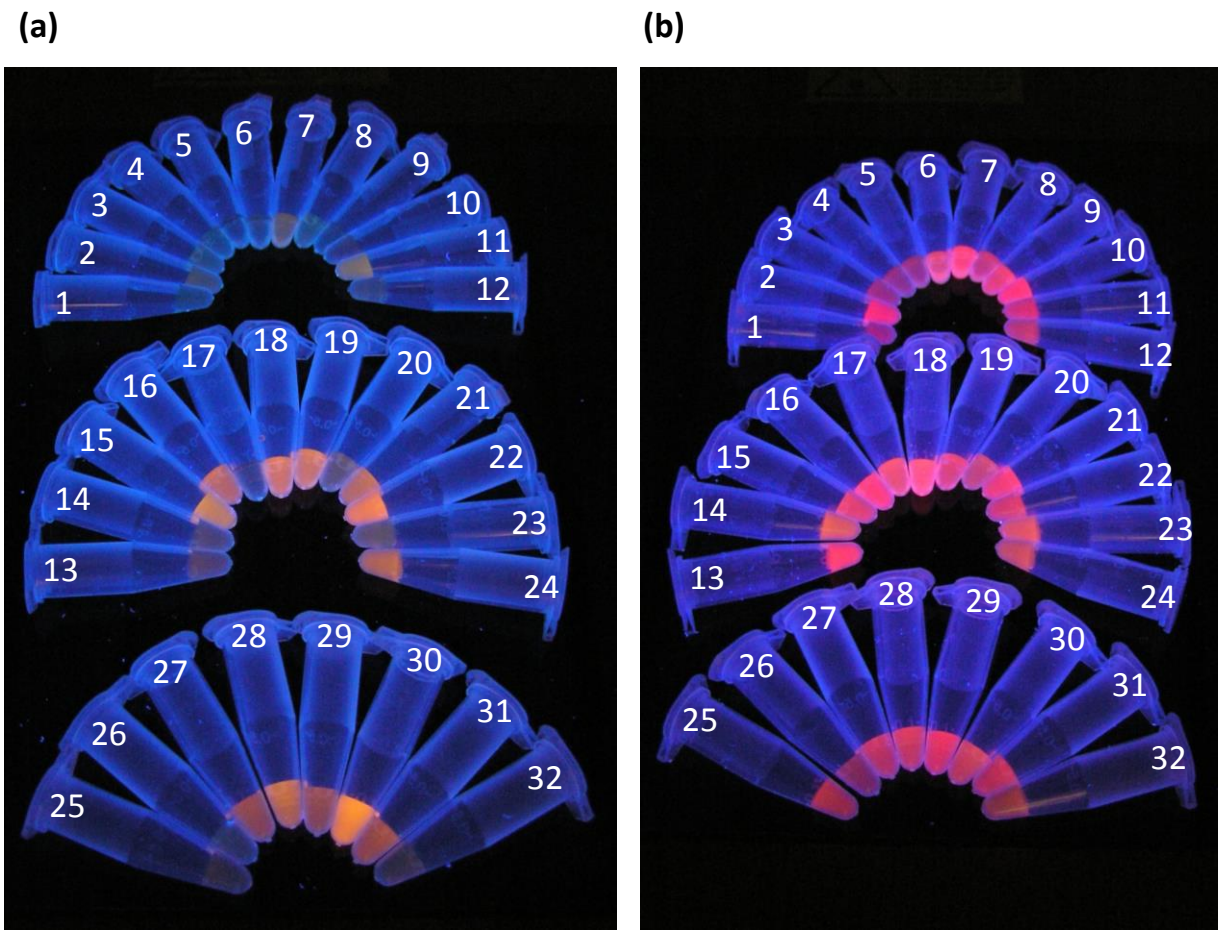


Figure S9. Color photos of 32 NCBs. Photos were taken under 365 nm excitation **(a)** before and **(b)** after activation.

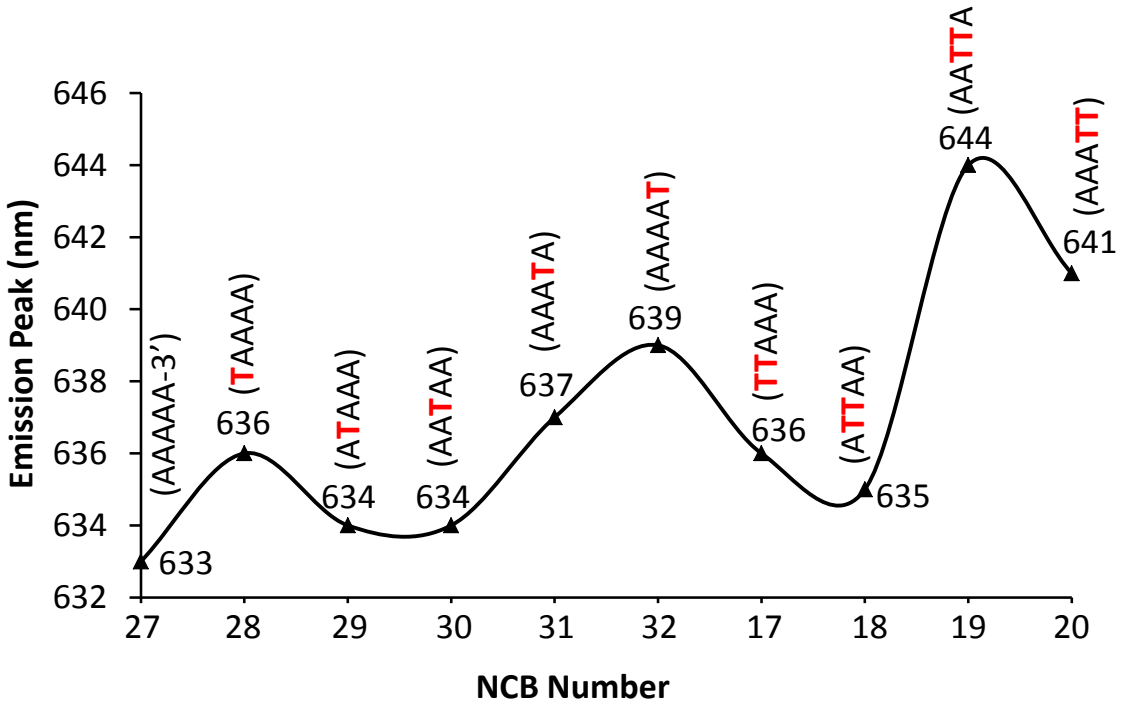
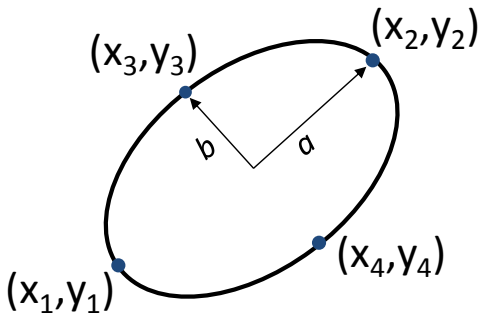


Figure S10. Emission peaks of selected NCBs. Here the excitation wavelength was fixed at 580 nm. In general, when the thymine sits right next to the polycytosine heads (*e.g.* No. 28 and 32 NCBs), thymine's "emission red-shifting power" is the strongest.

III. Symmetry on NCB's Spectral Profile

The degree of symmetry of a NCB's spectral profile was characterized by determining the eccentricity of the obtained 2D contour plot, which was fitted with an ellipse using Image J 1.48v (National Institutes of Health, USA) software. The eccentricity calculation is shown below:



The eccentricity (E) was calculated as:

$$E = \sqrt{(a^2 - b^2)/a^2}$$

where *a* and *b* are as follows:

$$a = \frac{\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}}{2}$$

$$b = \frac{\sqrt{(x_3 - x_4)^2 + (y_3 - y_4)^2}}{2}$$

In this report, we defined a symmetric spectral profile as E < 0.66.

Reference:

(1) Yeh, H.-C.; Sharma, J.; Han, J. J.; Martinez, J. S.; Werner, J. H. A DNA-Silver Nanocluster Probe that Fluoresces Upon Hybridization. *Nano Letters* **2010**, *10*, 3106-3110.